SAP Report No. 2001-09

REPORT

FIFRA Scientific Advisory Panel Meeting, July 17-18, 2001, held at the Sheraton Crystal City Hotel, Arlington, Virginia

A Set of Scientific Issues Being Considered by the Environmental Protection Agency Regarding:

Assessment of Additional Scientific Information Concerning StarLink™ Corn

NOTICE

This report has been written as part of the activities of the Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP). This report has not been reviewed for approval by the United States Environmental Protection Agency (Agency) and, hence, the contents of this report do not necessarily represent the views and policies of the Agency, nor of other agencies in the Executive Branch of the Federal government, nor does mention of trade names or commercial products constitute a recommendation for use.

The FIFRA SAP was established under the provisions of FIFRA, as amended by the Food Quality Protection Act (FQPA) of 1996, to provide advice, information, and recommendations to the Agency Administrator on pesticides and pesticide-related issues regarding the impact of regulatory actions on health and the environment. The Panel serves as the primary scientific peer review mechanism of the EPA, Office of Pesticide Programs (OPP) and is structured to provide balanced expert assessment of pesticide and pesticide-related matters facing the Agency. Food Quality Protection Act Science Review Board members serve the FIFRA SAP on an ad-hoc basis to assist in reviews conducted by the FIFRA SAP. Further information about FIFRA SAP reports and activities can be obtained from its website at http://www.epa.gov/scipoly/sap/ or the OPP Docket at (703) 305-5805. Interested persons are invited to contact Larry Dorsey, SAP Executive Secretary, via e-mail at dorsey.larry@.epa.gov.

CONTENTS

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Mr. Paul Lewis
Designated Federal Official
FIFRA Scientific Advisory Panel
July 25, 2001

Stephen Roberts, Ph.D.
FIFRA SAP Session Chair
FIFRA Scientific Advisory Panel
July 25, 2001

Federal Insecticide, Fungicide, and Rodenticide Act
Scientific Advisory Panel Meeting
July 17-18, 2001

Assessment of Additional Scientific Information Concerning StarLink™ Corn

PARTICIPANTS

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Dean Metcalfe, M.D., National Institutes of Health, National Institute of Allergy and Infectious Diseases, Laboratory of Allergic Diseases Bethesda, MD
Hubert P.J.M. Noteborn, Ph.D., State Institute for Quality Control of Agricultural Products AE Wageningen, The Netherlands
Nu-May Ruby Reed, Ph.D., California EPA, Department of Pesticide Regulation, Sacramento, CA
Marc Rothenberg, M.D. Ph.D., Children's Hospital Medical Center, Division of Pulmonary Medicine, Allergy/Immunology, Cincinnati, OH
Hugh Sampson, M.D., Mt Sinai/NYU Medical Center, Department of Pediatrics, New York, NY

PUBLIC COMMENTERS

Oral statements were made by:
Mr. James Chen, Hogan and Hartson, representing the American Seed Trade Association
Mr. Clint Krislov and Mr. William Bogot, representing Krislov and Associates, Ltd.
Mr. Larry Bohlen, representing Friends of the Earth
Ms. Grace Booth, private citizen
Anne Bridges, Ph.D., representing the American Association of Cereal Chemists
Mr. Charles Connner, the Corn Refiners Association, Clausen Ely, Jr., Covington and Burling, and Dirk Rief, Cargill Incorporated, representing the Corn Refiners Association
Mr. Will Duensing, Bunge Lauhoff Grain Company, representing the North American Millers' Association
Keith Finger O.D., private citizen
Mr. Bill Freese, Friends of the Earth, representing Genetically Engineered Food Alert
Rebecca Goldberg, Ph.D. representing Environmental Defense
Michael Hansen, Ph.D., representing Consumers Union
Susan Hefle, Ph.D., University of Nebraska
Mr. Don Hutchens, representing the Nebraska Corn Board and the National Corn Growers Association
Leah Porter, Ph.D., representing the American Crop Protection Association
Jupiter Yeung, Ph.D., representing the National Food Processors Association

Written statements were received
American Association of Cereal Chemists
American Crop Protection Association
American Seed Trade Association
Aventis CropScience
Environmental Defense
Keith Finger O.D., private citizen
Friends of the Earth
Novigen Sciences, Inc.
Larry Williams, Ph.D., Duke University Medical Center
National Food Processors Association
Nebraska Corn Board and the National Corn Growers Association
North American Millers Association
Mr. Larry Sallee, private citizen

INTRODUCTION

The Federal Insecticide, Fungicide, and Rodenticide Act (FIFRA), Scientific Advisory Panel (SAP) has completed its review of the set of scientific issues being considered by the Agency pertaining to an assessment of additional scientific information concerning StarLink™ corn . Advance notice of the meeting was published in the *Federal Register* on June 13, 2001. The review was conducted in an open Panel meeting held in Arlington, Virginia, July 17-18, 2001. The meeting was chaired by

Stephen Roberts, Ph.D. Mr. Paul Lewis served as the Designated Federal Official. Welcoming and opening remarks were preformed by Mr. Andrew Privee (Acting Associate Director, Office of Science Coordination and Policy, EPA) and Ms. Marcia E. Mulkey (Director, Office of Pesticide Programs), respectively. In addition, Patricia Hansen, Ph.D. (Deputy Director, Office of Science, Center for Food Safety and Applied Nutrition, Food and Drug Administration) provided remarks and clarified FDA comments at the meeting.

The following presentations occurred at the meeting:

- Introduction, goals and objectives - Janet Andersen, Ph.D. (Office of Pesticide Programs, EPA) and Ms. Laurel Celeste (Office of General Counsel, EPA)
- **Containment of StarLink™ corn on the farm** - Mr. Steve Gill (Farm Service Agency, USDA)
- **Purchase program for seed corn containing Cry9C protein -** Mr. Steve Gill (Farm Service Agency, USDA)
- **USDA detection methods verification and testing program** - Mr. Steven N. Tanner (Grain Inspection, Packers and Stockyards Administration, USDA)
- **Update on adverse event report -** Karl Klontz, M.D. (Food and Drug Administration)
- **Field epidemiological investigation of adverse event reports -** Carol S. Rubin, D.V.M., M.P.H. (National Center for Environmental Health, Centers for Disease Control and Prevention) and Richard Raybourne, Ph.D. (Food and Drug Administration)
- **Possible presence of Cry9C protein in processed human foods made from food fractions produced through the wet milling of corn** - Michael Watson, Ph.D. (Office of Pesticide Programs, EPA) and Mr. William Jordan (Office of Pesticide Programs, EPA
- **Performance of new method for detection of Cry9C protein in processed human foods** - Mary Trucksess, Ph.D. (Food and Drug Administration)
- **AventisCrop Science presentation**
  - Introduction - Mr. Richard Merrill (Covington and Burling)
  - Levels of Cry9C protein in foods made from 100% StarLink™ corn - Ms. Susan MacIntosh (Aventis CropScience)
  - Revised exposure assessment - Barbara Petersen, Ph.D. (Novigen Sciences, Inc.)
  - Concluding remarks - Mr. Richard Merrill (Covington and Burling)
- **Presentation of Agency questions** - Stephanie Irene, Ph.D. (Office of Pesticide Programs, EPA)

In this report, Cry9C refers to the protein while cry9c refers to the DNA molecule. In preparing this report, the Panel carefully considered all information provided and presented by the Agency presenters, as well as information presented by public commenters. This report addresses the information provided and presented within the structure of the charge by the Agency.

CHARGE

Exposure to Cry9C in Human Food:

At the November 2000 SAP meeting, EPA provided an exposure assessment for StarLink™ corn that was based on then available information. Specifically, EPA prepared an upper bound estimate of potential exposure to Cry9C protein as the consequence of the presence of StarLink™ in the human food supply. EPA used data on the consumption of food containing or made from corn, and data measuring the levels of Cry9C protein in corn grain. Notably, EPA assumed that the level of StarLink™ in finished foods was not reduced by processing of grain or by subsequent cooking.

EPA's exposure calculations also used information on the extent of planting of StarLink™ in 1999 and 2000, as well as information on grain handling practices, to produce high end estimates of the amount of StarLink™ corn that could be commingled with non-StarLink™ corn. These estimates did not take into account the various steps that have been taken since the fall of 2000 to limit the amount of Cry9C protein that could be present in the human diet. These steps include:

- the cancellation of the registration of StarLink™, and the prohibition of future planting of stocks of StarLink™ seed;
- the Aventis Crop Science - USDA program to purchase StarLink™ corn (and any corn commingled with it) and to direct such corn to animal feed or industrial uses; USDA's program to assure that non-StarLink™ seed for the 2001 corn crop that tests positive for the Cry9C protein not be sold for planting; and
- the efforts of corn handlers, millers, and food processors to assure that corn grain is tested for the possible presence of the Cry9C protein and that quantities testing positive are redirected away from the human food chain.

In addition, since November, more refined data and new analyses have been developed to estimate the amount of the Cry9C protein that would be available in finished food products. A new analytical method has been developed by EnviroLogix that is capable of measuring the levels of Cry9C protein in finished foods. Aventis has also provided data on the impact of processing and cooking on the levels of Cry9C protein in various types of finished foods made from corn. Aventis has employed these data to produce new estimates of potential exposure to Cry9C protein. Finally, EPA has prepared its own estimates of exposure to Cry9C protein in human foods made from the wet milling of corn.

1. The performance of the EnviroLogix ELISA test for the determination of Cry9C protein processed corn-based foods was evaluated using eight types of corn-based foods in an interlaboratory study involving seven laboratories in the United States. The FDA report on the multi-laboratory validation of the new analytical method indicated that the method is applicable to the determination of the Cry9C protein in eight types of corn-based products at levels equal to or greater than 2 ppb. In light of this assessment, please comment on the utility of this method for assessing the concentration of Cry9C protein in

processed corn based foods and specifically the use by Aventis of this method for their revised exposure estimates.

2. EPA has prepared a paper evaluating the impact of wet milling on the levels of Cry9C protein in human food products. Please comment on the levels of exposure to the Cry9C protein in the human diet likely to be encountered in food products as consequence of using human food fractions made from the wet milling of corn.

3. At the November 2000 meeting, the SAP reviewed the exposure assessment submitted by Aventis which estimated the possible levels of Cry9C protein that could be consumed by people eating food products made from corn if the corn contained any Cry9C protein. Aventis has submitted a revised exposure assessment which takes into account the new data estimating the levels of Cry9C protein that could survive processing, and thus occur in corn-based food products. Please comment on whether this updated assessment fairly and accurately depicts the levels to which consumers may be exposed.

4. Assuming the measures taken to limit the amount of StarLink™ in the human food supply are continued and with your knowledge of how corn and food products made with corn move through the channels of trade, please comment on the duration and levels of detectable amounts (at ppb) of the Cry9C protein that are expected to be in the human food supply from:
a) StarLink ™ corn planted in 1998 through 2000; and
b) From other domestic sources that might contain the Cry9C protein, e.g., volunteer StarLink ™ corn and non-StarLink™ varieties that express the Cry9C protein.

Allergenic Hazard and Risk:

The potential for the Cry9C protein to elicit an allergic response has been the single human health endpoint of concern for StarLink™ corn. In its December, 2000 report to the Agency, the Scientific Advisory Panel (SAP) concluded that "... there is a medium likelihood that the Cry9C protein is a potential allergen..." The SAP went further to recommend a number of follow- up activities that would allow for a better informed characterization of the potential allergenic risk. These activities included: (1) collection of data on the presence of specific antibodies in individuals either who claim to have experienced adverse effects after consuming food that might have contained the Cry9C protein or who have significant occupational exposure to StarLink™ corn or corn products, and (2) monitoring of reports from the medical community for individuals who claim to have experienced adverse effects either after consuming food that might have been made from StarLink™ corn or from occupational exposure to StarLink™ corn.

- FDA and CDC have been working together to investigate the adverse event reports submitted to FDA by people who claim to have had an allergic response following the ingestion of genetically modified corn products. One aspect of the investigation was to determine if these people were exposed and displayed an allergic response by the formation of serum antibodies to the foreign Cry9C protein. An FDA laboratory developed an enzyme linked immunosorbent assay (ELISA) method to detect these

antibodies in the sera of the people who were potentially affected. Although there were no known Cry9C-allergic human serum samples to serve as true positive controls, the assay was able to detect reactions in sera from goats that had been purposefully sensitized against the Cry9C protein, and also to detect reactions to certain human allergens (e.g., cat, grass, peanut) in sera from humans with known allergies to these allergens.

- Some of the individuals who claimed to have experienced an allergic reaction to the Cry9C protein following the ingestion of corn-based products kept samples of (or could identify) the products they ingested. FDA tested these foods for the presence of StarLink™ corn. StarLink™ corn DNA has not been detected in 10 of 11 food samples analyzed using the PCR method. The other sample of food, which tested positive using the PCR method, was not from the consumer's actual product, but from a different lot of the same product collected by FDA from a grocery store. In addition, the Cry9C protein was not detected is 9 (including the food sample that tested positive using the PCR method) of the 10 samples tested with the EnvioLogix ELISA method. One of the 10 samples tested using the EnviroLogix method was inconclusive. There was no testing of one food sample using the EnviroLogix method because there was not enough of the remaining sample to conduct the test.

Given these circumstances, please comment on:

a) the ability of the test to detect Cry9C-specific antibodies;

b) the criteria used to designate test results as positive or negative, and the significance of positive and negative results obtained using this test;

c) the ability of the test to either identify or eliminate Cry9C as a potential cause of the allergic symptoms reported; and

d) the usefulness of the test, along with other information gathered in the FDA and CDC investigation, in evaluating whether an individual has experienced an allergic reaction to the Cry9C protein.

6. In the December, 2000 SAP report, after reviewing the information then available concerning the Cry9C protein, the Panel concluded that "... there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of the Cry9C protein itself..." The same report went on to state that "Given the current state of knowledge regarding allergens and the uncertainties of ascertaining the exact amounts of Cry9C in the food chain, this approach [collecting data on the presence of specific antibodies in individuals claiming exposure to Cry9C in food products] could provide 'hard evidence' as opposed to speculation on the question at hand." Since then, additional information concerning the potential allergenicity of the Cry9C protein has become available, including the FDA/CDC report issued on June 11, 2001, which provides information on the presence of Cry9C-specific antibodies in individuals claiming to have experienced an allergic reaction after eating corn-based foods. In light of the available information, what is the current Panel's view on the previous finding of

that there is a "medium likelihood" that Cry9C protein is a human allergen? Please comment specifically on whether and how that view is significantly affected by your consideration of the June 11, 2001 reports from FDA and CDC.

7. In its December 1, 2000, report, the Panel concluded that "...the likely levels of Cry9C protein in the U.S. diet provide sufficient evidence of a low probability of allergenicity in the exposed population."

a) In light of the new information on the levels of Cry9C protein in the diet and the other available information concerning potential allergenicity, please comment on the overall probability that the likely levels in the US diet of Cry9C protein are sufficient to cause significant allergic reactions in a major identifiable subgroup of the exposed population. To the extent permitted by available information, please characterize the current level of potential risk in terms of the proportion of the population likely to be affected and the nature and severity of potential effects.

b) If you conclude that it is probable that the expected levels of Cry9C protein are sufficient to cause significant allergic reactions in a major identifiable subgroup of the exposed population, please identify a level of Cry9C protein below which you would not expect significant reactions to occur in a major identifiable subgroup of the exposed population.

c) Based on your responses to questions 7 a) and 7 b), do you conclude that there appears to be a maximum level of Cry9C protein for which, if that level were found in corn grain and foods made from such grain, there would be a reasonable scientific certainty that exposure would not be harmful to public health? Please explain your answer.

Possible Need for Additional Data and Additional Public Health Measures:

8. In its December 2000 report, the SAP concluded "...the Agency should place ...priority on monitoring of reports from the medical community. The Panel felt that the medical community should be informed of the investigation into the allergenicity of Cry9C in corn products." Approximately 8 months have passed since that original recommendation and, given the materials that have been discussed at today's meeting, we ask the Panel to please comment on the value of implementing a program involving the medical community intended to detect instances in which individuals experienced allergic reactions to the ingestion of Cry9C protein in food. If the Panel still regards such a program as potentially valuable, then please comment on the scope and design of such a program.

9. In its December 2000 report, the SAP identified additional types of information that could improve EPA's ability to assess the potential allergenic risk to humans from Cry9C protein in the food supply. In response to the Panel recommendations, Aventis Crop Science and the Federal government have developed new information on the Cry9C protein which has been presented to the Panel today. Given all the information that we presently have, please characterize generally the adequacy of the existing scientific

database to evaluate the allergenic risk of Cry9C and identify any additional information that would be feasible to generate and would be likely to change significantly the current assessment of the allergenic risk to humans from the Cry9C protein in the food supply.

10. From a public health perspective, please identify other measures, if any, beyond those currently being implemented that you consider feasible and necessary to reduce the likelihood that people would experience allergic reactions from ingestion of food containing Cry9C protein.

11. Are there any other comments on the science of this issue that EPA should consider or that the SAP panel would like to address?

DETAILED RESPONSE TO THE CHARGE

The specific issues to be addressed by the Panel are keyed to the Agency's background document "Transmission of Background Document for the FIFRA Science Advisory Panel Entitled *Assessment of Additional Scientific Information Concerning StarLink Corn*," dated July 3, 2001, and are presented as follows:

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The EnviroLogix ELISA test appears to be a sensitive, reproducible procedure as indicated in the collaborative study. However, there appears to be a major problem that probably involves the solubility of the protein after cooking. The critical question involves whether the solubility of the Cry9C protein or its fragments, after heating, extrusion or other processing steps, will still allow detection by the ELISA system being used. This must be resolved in order for this ELISA test kit to be validated for detection of Cry9C protein in processed foods. If the EnviroLogix ELISA fails to detect all relevant forms of the Cry9C protein, use of analytical results obtained from this procedure can result in underestimating the amount of Cry9C protein in the case of food products obtained from ingredients derived from dry milling or the masa process. Until this is investigated, this ELISA procedure is of questionable value for developing exposure estimates for processed foods. It is recommended that the Cry9C protein content of food products made from dry-milled and masa-process ingredients be based on the Cry9C protein content of corn meal, corn flour, or masa dough.

This conclusion is based on the following considerations. FDA evaluated the accuracy and precision of the EnviroLogix ELISA test kit as a potential method for the detection of Cry9C protein in processed corn-based foods. The assay is a double antibody sandwich format and is based on the specific interaction between antibody and antigen.

The performance of the test kit was evaluated using eight types of corn-based foods (starch, refined oil, soft tortillas, tortilla chips, corn flakes, corn puffs, corn muffins, and corn bread). The interlaboratory, collaborative study involved seven laboratories in the United States. While this does not fully meet the Association of Analytical Chemists (AOAC) and American Association of Cereal Chemists (AACC)

criteria for collaborative studies, it is sufficiently stringent to establish the accuracy and precision of the methodology. The AACC is currently conducting a collaborative study of this method using 26 laboratories in 12 different countries. Data from this study are being analyzed and the method is expected to be ready for the first approval status step in October, 2001 (Testimony by Anne Bridges, EPA SAP 7/17/01).

In the FDA collaborative study, Cry9C protein from two different sources was used to spike the food products. Cry9C protein produced and purified from *E. coli* was used to spike test samples at 2.72 and 6.8 ng/g (ppb). Cry9C protein from StarLink™ corn flour was used to spike test samples at 1.97 ng/g. FDA reported that the method is applicable to the determination of Cry9C protein in eight types of corn-based products at levels ≥2ng/g (2 ppb)

The utility of this procedure to assess the concentration of Cry9C protein in certain processed corn-based foods is still open to question. As reported in Aventis Report No. CM00B014, pages 32-33, "The loss of Cry9C protein is due to a combination of recipe dilution, processing methods and cooking. The greater the dilution and the more harsh the processing/cooking (heat, shear or pressure and alkali treatment), the lower the level of Cry9C protein in the finished food product." The harsh treatment referred to above will reduce the level of Cry9C protein as determined by the ELISA procedure. However, this may simply be because the Cry9C protein has been rendered insoluble in the solvent used in the ELISA procedure or is no longer recognized by the antibody due to change in tertiary structure or molecular size. The protein is not destroyed and is still in the product in some form. It may be denatured or bound to some other constituent. The cooking conditions used are not likely to hydrolyze the protein to amino acids or cause it to vaporize. It is conceivable that the insoluble Cry9C protein or its fragments might still have allergenic potential.

The problem with loss of detectable protein is clearly illustrated in analytical results reported by Aventis (Report No. B003244, Figure 5, page 43). Corn bread retained 15.4% of the Cry9C protein originally present in the corn meal/corn flour made from 100% StarLink™ corn while corn muffins retained only 5.2%. The only significant difference between these two products is that one is baked in an eight-inch pan while the corn muffins were baked in a muffin pan, i.e. a difference in the surface:volume ratio. Protein is not destroyed under these conditions. Rather, it may be denatured or otherwise transformed to a form that is not detected by the EnviroLogix ELISA analytical system.

The effect of recipe dilution is real and must be considered. In processes such as masa production, it would appear that much of the Cry9C protein is extracted into the steep water and, thereby, washed away. This results in relatively low levels of Cry9C protein in the products made from masa. However, the losses occurring by the baking and/or frying of the masa must be questioned. The same is true for losses that occur during the cooking of products made from dry milled fractions. The pertinent method would appear be to consider that products such as corn muffins, corn bread, corn curls, etc. contain the recipe dilution level based on the level of Cry9C protein in corn meal or flour and ignore the apparent losses caused by cooking.

Concerns were also raised by the Panel concerning the general use of the EnviroLogix ELISA system for this analysis. The detection antibody was raised to bacterial Cry9C protein rather than to the corn-derived protein. For the procedure to be accepted, it must be unequivocally shown that the two proteins are the same. A recurring question, for which there are no definitive data, concerned whether all denatured and or degradation products of the Cry9C protein are being recognized. This is a critical concern for this analytical system.

2. EPA has prepared a paper evaluating the impact of wet milling on the levels of Cry9C protein in human food products. Please comment on the levels of exposure to the Cry9C protein in the human diet likely to be encountered in food products as consequence of using human food fractions made from the wet milling of corn.

The Panel concluded that the EPA used a reasonable approach in determining human dietary exposure from corn starch produced from Cry9C protein containing corn via the wet milling process. The vast majority of Cry9C protein of corn processed by wet milling will be found in the animal feed products including bran, gluten feeds, steep liquors, to a very limited extent in starch and not in oil products or products produced from starch. A detailed summary of the Panel's conclusion is provided below.

The wet milling process is done to separate the protein and non-protein fractions of corn. As a result of this processing, no Cry9C protein was detected in refined corn oil, alcohol, corn syrup and dextrose, high fructose corn syrup and crystalline fructose made from corn syrup, as analyzed by several different laboratories using the EnviroLogix ELISA kit (EPA-White Paper on the Possible Presence of Cry9c Protein in Processed Human Foods Made from Food Fractions Produced Through the Wet Milling of Corn; Aventis report CM00B014-EPA #B003244; testimony from Mary W. Trucksess, FDA, to the SAP on 7/17/01). Further, the DNA coding for the Cry9C protein was not detected in maltodextrin, glucose syrup, crystalline dextrose, crystalline fructose or corn oil. The Aventis analysis of refined oil from corn containing 14,275 ppb Cry9C protein showed no detectable protein when analyzed with the EnviroLogix ELISA kit with a limit of detection of 0.35 ppb. This same Aventis report on the Cry9C protein in wet milled products derived from 100% StarLink™ grain showed 13.2 ppb Cry9C protein in corn starch as determined by the EnviroLogix ELISA procedure. The corn starch analyzed in this case was produced in a Texas A&M pilot processing plant.

The Aventis analysis for Cry9C protein using EnviroLogix ELISA procedures was nearly equivalent to the EPA estimates. However, the Panel noted that the Aventis starch samples processed in the pilot plant contained 0.5% protein, much higher than 0.30-0.35% typically found in starch produced in commercial plants. The low levels of protein in corn starch are the result of separation of the germ and numerous water washes to remove water-soluble proteins. Cry9C protein is a water-soluble protein. Clearly, the corn starch produced in the pilot plant was not as low due to less efficient starch removal than in commercial plants. Thus, the Aventis analysis of pilot plant processed starch resulted in estimated exposures higher than products from commercial plants.

Based on EPA estimates of Cry9C protein exposure from starch that assume the use of strip tests to assure <0.125% Cry9C protein containing corn is used in wet mill processing, upper bound estimates of per capita daily exposure would be 0.0013161 ug for the U.S. population at the 99.5 percentile. If the source corn was 1.5% or 1.2% Cry9C protein (assume no screening with EnviroLogix or SDI strip tests), the upper bound estimates for per capita daily exposure are 0.01959 and 0.01567 ug, respectively, at the 99.5 percentile. These values are based on the Aventis data indicating 13.2 ppb Cry9C protein in pilot plant processed corn starch. The EPA White paper document using the USDA 1989-1991 Continuing Survey of Food Intakes by Individuals (CSFII) also makes calculations for infants and children, with exposures approximately one order of magnitude less for total exposure than the entire US population. The Agency White paper, citing industry sources, indicated that approximately 33% of the corn processed for the food starch market is waxy maize, a type of corn for which varieties with the Cry9C protein have not been produced. Thus, EPA may be overestimating exposure by as much as 33%.

The Panel noted that one protein product, corn zein, is occasionally produced in corn wet mills. This product is used in glazing and coating agents for the food and pharmaceutical industries. This is a water insoluble protein and high volumes of water are used in its purification. This processing technique should eliminate the water soluble Cry9C protein in much the same way as in starch production. No Cry9C protein analytical data are available for this product.

The Panel provided the following recommendations to refine the Agency's dietary exposure assessment.

(1) Similar to the Panel's recommendation at the November, 2000 SAP meeting, dietary exposure should also be presented on a per user basis. The per capita profile may not be very different from the profile of per user if the commodity is widely consumed (i.e., having high percentage of users per surveyed population). However, if the percentage of users in a surveyed population is low, the exposure distribution will be shifted to the lower level and will have different values at a given percentile. In the corn starch analysis case, this information is not given.

(2) EPA indicated that instead of using the more recent CSFII data, the 1989-1991 CSFII data were used in the exposure analysis because the recent CSFII data were not available to the Agency at the time the exposure assessment was conducted. If the 1994-1996 CSFII and the 1998 children subset are available to the Agency for corn starch exposure, it is recommended that separate exposure analyses using the latter data should also be presented to provide a more robust picture of the exposure, especially to a major identifiable subgroup (i.e. children). The Panel recognized that the use of more recent consumption data may not substantially alter the exposure estimates. The 1994-1996 CSFII database was used by Novigen Sciences, Inc. (on behalf of Aventis CropScience) in their report "Estimated Potential Dietary Intake of Cry9C Protein in Processed Foods Made from 100% Starlink™ Corn" completed 4/12/2001, and the exposure estimates are similar to those in the EPA White paper. The Novigen Sciences, Inc., report covers all

potential food intakes and does not allow examination of exposure from corn starch exclusively.

3. At the November 2000 meeting, the SAP reviewed the exposure assessment submitted by Aventis which estimated the possible levels of Cry9C protein that could be consumed by people eating food products made from corn if the corn contained any Cry9C protein. Aventis has submitted a revised exposure assessment which takes into account the new data estimating the levels of Cry9C protein that could survive processing, and thus occur in corn-based food products. Please comment on whether this updated assessment fairly and accurately depicts the levels to which consumers may be exposed.

Concerns were raised about the accuracy of the revised Aventis dietary exposure assessment for Cry9C protein based upon our evaluation of the available information. This is particularly true because of the Panel's concern about the appropriateness of the analytical method used to determine Cry9C protein content in finished food as described in Question 1.

Dietary exposure is a function of the amount of a substance in foods as consumed and the consumption rate of those foods. An accurate and conservative dietary exposure assessment for Cry9C protein should be based upon the best available and scientifically defensible data on Cry9C protein in finished foods, the prevalence of StarLink™ grain in the U.S. food supply, the corn protein content of finished foods, and consumption of finished foods by the U.S. population. In addition, the results of the assessment should be presented in an accurate and transparent manner. A detailed review of Aventis' revised submission is provided below.

Overview of the Revised Aventis Dietary Exposure Assessment

Aventis has revised the exposure assessment reviewed at the November 2000 SAP meeting by making the following changes:

- Actual measurements of Cry9C protein in 10 finished corn-based foods were used in place of estimates based on the protein content of corn grain;
- Corn starch was included as a potential source of dietary exposure of Cry9C protein using the values measured in the Aventis processing study;
- StarLink™ was assumed to constitute 0.125% of corn in the U.S. food supply, which corresponds to the 20 ppb limit of detection of the Lateral Flow Strip Test used in milling facilities.

Exposure is calculated as the consumption of foods that could contain Cry9C protein multiplied by the level of Cry9C protein in the foods and the fraction of StarLink™ corn in the grain stock. The consumption database is taken from the 1994-1998 CSFII data. The high end exposure is presented as the 99th percentile of the per user exposure distribution. The content of Cry9C protein in foods was taken from the Aventis report CM00B014 (Shillito et al., Volume 5 of 7). The use of surrogate data

when data on Cry9C concentration was not available appears to follow reasonable and conventional practices. This approach appears to be similar to that used by USEPA in calculating the exposure to Cry9C protein from corn starch consumption, except for the multiple sources of exposure that substantially complicated the analysis and that carried some important implications in the final result. The SAP opinion on dietary exposure to Cry9C protein from wet milled corn products such as corn starch may be found in the response to Question 2. The revisions contained in the new dietary exposure assessment raise several issues that deserve consideration.

Accuracy and Fairness of the Revised Aventis Dietary Exposure Assessment

Cry9C Protein Content of Finished Foods

An accurate exposure assessment requires that the Cry9C protein levels measured in finished foods are accurate, precise, and representative of a wide range of corn-based foods. As indicated above, the measured levels of Cry9C protein in finished foods are described in Aventis report CM00B014 "Detection of Cry9C protein in dry milled, wet milled and masa processed fractions and processed foods made from 100% StarLink grain". In this experiment, raw StarLink™ corn was wet milled, dry milled, and masa processed to produce a suite of corn product intermediates including starch, gluten, hull, oil, corn meal, corn flour and masa dough among others. The intermediates were used to produce 10 finished foods: tortillas (soft), tortilla chips (fried), corn puffs, ringed cereal, corn flakes, taco shells (baked), polenta, hush puppies, corn muffins, and corn bread. Two ELISA methods were used to quantify concentrations of Cry9C protein in each product and food, one developed in-house by Aventis and another developed by EnviroLogix Inc. The Cry9C protein concentrations used in the dietary exposure assessment are based upon results of the EnviroLogix assay.

As described in the response to Question 1, the SAP had concerns about the validity of the EnviroLogix ELISA test with regard to detection of Cry9C protein after heating, extrusion, and other common food processing steps. Use of the processing experiment data based upon measurements made by the EnviroLogix test can result in underestimates of dietary exposure to Cry9C protein in finished foods produced from dry milled and masa processed corn intermediates. For this reason, the SAP is supportive of a dietary exposure assessment based upon the Cry9C protein content of corn meal and masa dough prepared from 100% StarLink™ grain and as determined by the EnviroLogix assay. Estimated upper bound exposure generated by use of Cry9C protein content of corn intermediates rather than the available finished food data could be several fold greater than the 0.37 ug/day presented in the revised Aventis dietary exposure assessment.

StarLink™ Grain in the U.S. Food Supply

The revised Aventis dietary exposure assessment report assumes that 0.125% of corn in all corn-based foods, including those produced from white corn, is StarLink™ grain. Most corn delivered to dry mills is contracted with corn producers by the mills.

Dry mills only process corn for human food use. Because of the split registration for StarLink™ corn (i.e. feed use only), it is highly unlikely that dry mills will receive corn containing StarLink™ grain. According to testimony by the North American Millers Association at the July, 2001 SAP meeting, only 1.2% of 85,000 truckloads of corn received by dry millers was found to test positive for StarLink™. There appears to be a high rate of compliance with recommendations for testing incoming grain in the food corn processing industry (see Question 4). Loads of corn in which Cry9C protein is detected are likely to be rejected. For these reasons, the SAP believes that the assumption of 0.125% StarLink™ corn in the food supply is highly conservative. Detailed comments on this subject are provided in the SAP response to Question 4.

Food Composition Tables and Food Consumption Data

Reporting exposure at only fixed percentiles as done in the revised Cry9C protein exposure assessment leaves some questions unanswered. One example is the sufficiency of the current report in expressing the high end exposure. This comment is illustrated in the following simple calculation for corn bread consumption. Using the same parameter values in the Aventis analysis for the Cry9C protein residue (i.e., 2316 ppb) and StarLink™ mixing factor (0.125%), the 0.37 ug/day exposure represents consumption of less than 5 oz (approximately 140 g) of corn bread as the sole source of Cry9C protein exposure. This consumption rate is estimated to be approximately two pieces of corn bread, and cannot be considered as an excessive or high end of consumption for an adult. It should be noted that this estimation is based on the consumption of corn bread alone, without the many other possible sources of corn products that could add to this person's exposure to Cry9C protein, such as a bowl of corn cereal in the morning or a couple of tortillas for lunch, with the corn bread eaten at dinner. Therefore, the Aventis exposure assessment as expressed at the 99th percentile does not appear to reflect the high end of exposure.

The exact reason for the apparent deficiency for defining a high end of exposure cannot be identified without further information on the Novigen proprietary software used to perform the assessment. One possible reason could be the dilution of the population exposure profile. When foods or ingredients that have high frequency of consumption but low level of exposure are included in an analysis (e.g., corn starch), they tend to shift the exposure distribution toward the lower level due to the increased population with relatively low exposure. A shift of this type can mask the exposure profile of high contributing foods or commodities (in this case, corn bread and corn muffin, with 57% contribution to the total exposure), especially when the results of an analysis are only expressed as a fixed percentile of exposure. In addition, when this percentile approaches an extreme value (e.g., 99th percentile), the assessment methodology may appear to be more conservative than it really is. In these cases, the context of statistical expression is crucial to the understanding of the outcome as expressed.

Moreover, the need to assure the representativeness of data used in the analysis cannot be overstated, especially that the general practice in an exposure assessment is to

be able to capture the reasonable high end value. Again, the exposure from the two pieces of corn bread is used for an illustration. The corn muffin/bread recipe in the McNair's Favorites cookbook used in preparing the StarLink™ corn bread and muffin called for a ratio of 1:1.12 for wheat flour and corn meal. A limited search of household recipe books showed a wide variation for this ratio, ranging from 1:0.5 to 1:1.7. Thus, using the recipe of 1:1.7 mix, a reasonable adult exposure could be further raised by 41%.

Uncertainty Analysis

A balanced and comprehensive analysis and discussion of scientific uncertainty about input parameters and methods used to perform the analysis is required to assess the accuracy and fairness of an exposure assessment. Without such a discussion presented in the context of the analysis, it is impossible for a general reader to understand the meaning of these exposure estimates. As indicated above, limited information on Cry9C protein content of finished foods is an important source of uncertainty for assessment of dietary exposure to Cry9C protein. Accurate data on the amount of StarLink™ grain in the U.S. food supply, retrospectively and prospectively, is also an important source of uncertainty. In contrast, the SAP believes that the available data on protein content of foods as consumed (USDA food composition tables) and consumption of foods that contain corn protein (1994-1998 CSFII) are relatively certain.

4. Assuming the measures taken to limit the amount of StarLink™ in the human food supply are continued and with your knowledge of how corn and food products made with corn move through the channels of trade, please comment on the duration and levels of detectable amounts (at ppb) of the Cry9C protein that are expected to be in the human food supply from:
a) StarLink ™ corn planted in 1998 through 2000; and
b) From other domestic sources that might contain the Cry9C protein, e.g., volunteer StarLink ™ corn and non-StarLink™ varieties that express the Cry9C protein.

The Panel concurs with EPA's conclusion that based on the information presented by USDA, FDA, CDC, Aventis and the food industry, and assuming measures to isolate StarLink™ from the food corn supply are continued, the amount of Cry9C protein in the human food supply is significantly less than the estimates developed by EPA in November, 2000. Assuming a consistent program of testing grain entering food processing plants and reductions of Cry9C protein due to processing (as discussed in Question 2), the Panel concluded that the levels of Cry9C protein entering the U.S. food corn supply are very low. EPA estimated current concentrations of Cry9C protein in food corn to be 0.34 ppb, using the Aventis estimate of 5% rejection rate at corn dry mills (Brassard, 2001a). Based on the North American Millers Association (NAMA) industry data showing 1.2% rejection rate, the concentration in food corn samples could be as low as 0.1 ppb. Additionally, the concentrations of Cry9C protein in both general grain stocks and the US food corn supply will decline rapidly after the 2001 crop is harvested and with each subsequent production year. The Panel's conclusion is based on: reported success of the seed testing program, and the low percentage of Cry9C protein in cross

pollinated corn and other unintended production sources, and grain storage and corn carry over patterns.

This analysis was based on several factors which influence the estimated amount and duration of Cry9C protein in the food corn supply: (1) the amount of StarLink™ corn produced and marketed off-farm, (2) other potential sources of the Cry9C protein, (3) the impact of on-going testing programs, (4) success of containment programs and the degree to which data from grain testing programs support concentrations predicted from production and containment information, (5) grain storage and carry over patterns, which estimate the persistence of the Cry9C protein after intended production ceases, and (6) the impact of on-going testing programs by food processing firms. A detailed discussion of this analysis follows.

(1) StarLink™ Corn Production

Aventis reported the following planting of StarLink™ corn in 1998-2000 (EPA, 2000).

Year	**Acres**	**Percent of US Corn**
1998	9,000	0.01
1999	248,000	0.32
2000	341,000	0.43

The distribution of StarLink™ corn across the U.S. for crop year 2000 is known (Harl et al., 2000 with updates) but is unknown for previous years. In 2000, StarLink™ corn was grown across the corn production areas, with the highest concentration in Iowa.

Actions have been taken to control production of StarLink™ in the 2001 crop. In late December 2000, the USDA and state Extension specialists strongly recommended that seed companies test all of their 2001 seed corn lots and parent lines for the presence of the Cry9C protein (Nielsen and Maier, 2001). Any seed lot testing positive for the Cry9C protein was to be channeled by the seed company into feed or non-food industrial use, or destroyed. USDA also recommended that seed companies provide the verification information to customers. The seed industry has responded by testing seed lots for Cry9C protein (although not all companies were willing to provide public verification of results). It should be noted that, given the limitations of accuracy and precision of current test methods, seed companies cannot guarantee zero presence of the Cry9C protein in any seed lot. The USDA Farm Service Agency testified to the Panel that USDA was confident in the success of the seed containment program (Gill, 2001). Corn growers have been advised to retain the results from the USDA-recommended seed-testing plan for the Cry9C protein with the seed lot numbers for their records. Additionally, farmers received advice on controlling volunteer corn from 2000 StarLink™ production or buffer areas.

Based on these actions, it appears that the ongoing risk of new sources of Cry9C protein entering the food supply from the 2001 corn harvest has been managed. Aventis and its licensees through separate actions are overseeing the systematic destruction of the global StarLink™ seed inventory. Further commercial and breeding development of StarLink™ corn has been discontinued wordwide. Management advice regarding volunteer corn is being provided to growers and industry-wide verification procedures for 2001 corn seed are in-place. However, there has not as yet been provision for independent auditing to validate these claims.

(2) Other Potential Sources of Cry9C Protein

Other sources of Cry9C protein production include corn grown in buffer fields, other cross pollination of StarLink™ corn or corn seed, volunteer corn in corn or soybean fields, and unintended mixing in seed conditioning operations. There has been no evidence of Cry9C protein expression by non-StarLink™ varieties which have not been cross pollinated with StarLink™. Cross pollination occurs in buffer zones. Buffer production is defined by EPA regulations as production within 660 feet of StarLink™ planted acreage. Corn harvested from these acreages faced the same market limitations under the original StarLink™ label and is handled as StarLink™ corn under the containment program.

Pollination effects and the potential for cross pollination outside of the buffer zone are not well documented. Anecdotal evidence suggests long distance pollen travel is possible as in, for example, the recent findings of StarLink ™ in white corn (due to yellow StarLink™ kernel contamination) and the 3-5% of U.S. seed corn stock found to have unintended low level (<0.2%) StarLink™ presence. Volunteer corn in soybean fields is controlled with herbicide treatment. However, the additional Cry9C protein containing corn generated from unintended sources other than buffer fields is estimated at less than 1% of the total Cry9C protein containing corn production.

Reported buffer production was 43.9 million bushels for the 2000 crop. EPA reported that buffer corn expressed 1% of the Cry9C protein concentration as direct StarLink™ acreage (0.15 p.m. versus 12.9 ppm. (Brassard, 2001b)), a significant reduction from earlier estimates of up to 16%. Therefore, buffer crop and unintended production adds less than 2% to total Cry9C protein production.

The expression level of Cry9C protein across hybrids and environments is a source of error in Cry9C protein production estimates. Aventis reported that Cry9C protein was 0.013% of total protein (EPA, 2000). Subsequently, Hefle indicated that Cry9C protein content varies from 0.008% to 0.032% of protein across hybrids and environments (S. Hefle, University of Nebraska, personal communication). In oral testimony to the SAP, Aventis indicated a range of 0.010% to 0.020% of protein. Most composition factors are influenced by genetics and the environment, so that these variations are expected. Variability in Cry9C protein expression affects analysis methods and prediction of Cry9C protein produced. All assessments presented to the Panel were based on the average expression without regard for a possible range.

Recently a concern has arisen over the presence of the StarLink™ Cry9C protein in white corn products made from masa milling. Although FDA was unable to detect Cry9C protein, it did confirm the potential presence of *cry9c* DNA. Previously the Panel heard that masa production consumes about 60 million bushels annually, and that 48 million bushels are white corn where no transcend hybrids have been produced (EPA, 2000). Most food corn producers, handlers and processors use both white and yellow corns. Contractors specify the acceptable amount of yellow corn present in white corn contracts. They typically range from contract lows of 0.5-1% to highs of 3-5%, and farmers will receive a lower price for delivering white corn with higher levels. However, most food corn contractors specify hard yellow endosperm hybrids (compared to the soft endosperm hybrids used for StarLink™), and most do not have any Bt food corn hybrids on their approved hybrid lists. Thus, the potential sources for StarLink™ contamination of white corn are the same as for yellow food corn - seed impurity, pollen drift, and commingling during harvest, handling, transport and processing. The likelihood of StarLink™ in white corn lots remains very low, but not zero.

(3) Grain Testing Programs

Grain test results are potentially useful in judging Cry9C protein content of grain stocks in market channels. The Grain Inspection Packers and Stockyards Administration (GIPSA) lateral-flow strip test protocol (GIPSA, 2000) has been progressively modified (from one 400 seed sample to the current three 800 seed samples) to lower the detection limits and obtain consent of buyers. At the present sensitivity (0.125%), GIPSA is experiencing 23% positive results (GIPSA personal communication). Fewer positives were obtained at lower sensitivities. If the GIPSA data approximates general market conditions, where no variety control or selective buying program exists, then the general U.S. corn supply may contain more Cry9C protein than would be estimated from containment data. Approximately 100,000 inspections contributed to these data. However, the GIPSA data are not necessarily representative of grain inventories. They represent primarily tests of trucks and railcars bound for export, grain entering wet milling operations, and samples which for any reason grain handlers submitted to GIPSA. The submitted samples likely come from grain firms trying to confirm suspected lots of corn, in which case they would provide an upward biased estimate of Cry9C protein levels in the grain stream.

Fewer positive tests were reported by the corn dry milling industry (NAMA, 2001). Corn dry mills have limited trade areas and generally have selective buying practices for other quality reasons. Before the meeting, Aventis reported that dry millers were obtaining about 5% positive tests (Brassard, 2001a). NAMA reduced that estimate to 1.2% in testimony to the SAP, based on about 86,000 inspections at their plants. Incidence of StarLink™ at corn dry mills was approximately 20-25% of that in general market channels, with each positive detect load being rejected.

(4) Success of Containment Programs

Awareness of contamination of StarLink™ corn with corn intended for food began with the September 29, 2000 release of data on taco shells. Rapid test kits for StarLink™ were first available in official inspections on November 15, 2000 (GIPSA, 2000), by which time the majority of U.S. corn had been harvested. Between 30 and 50% of U.S. corn is moved to market at harvest; the remainder is stored on farm, for later delivery.

There are varying reports as to the amount of uncontrolled StarLink™ corn in U.S. corn supplies. In oral testimony to the November 28, 2000 SAP meeting, USDA stated that 7 million bushels (11%) of the StarLink™ were not accounted for. Gill (2001) stated that as of July 17, 2001 USDA estimates that there are 720,000 bushels of uncontrolled StarLink™ corn in elevators. Gadsby (2001) indicated that Aventis believes that none of the 2000 StarLink™ corn remained in the general market as of April 17, 2001. Grain handlers' opinions are that, at least in some regions, a significant fraction of corn in position for shipment is commingled with StarLink™, although quantitative estimates are not possible from this source.

There are several likely explanations for the variations in containment estimates. Contained amounts of corn grown from StarLink™ seed are not always clearly distinguished from amounts of blended StarLink™ and non-StarLink™ corn. The USDA producer reporting system was stated to be voluntary, not linked to sales records of corn seed, which means those producers choosing not to respond for whatever reason are not counted (Gill, 2001). Grain handlers themselves have had difficulty distinguishing volumes of StarLink™ mixtures (with unknown concentration) from amounts of pure StarLink™ corn.

Estimating Cry9C protein containing corn levels in U.S. corn stocks is impacted both by the sampling data used (different frequency of positives in the GIPSA and NAMA samples) and by the level of Cry9C protein assigned to the respective positive test results. Sampling errors are typically large, in the order of 25-50% in any binomial event based testing.

An intensive reevaluation of all data, tracking mass of Cry9C protein rather than percentages of corn, and resolving differences in evaluation of sampling data could improve the estimates of Cry9C protein in U.S. corn stocks. This would also assist in planning for future registration of transgenic plants. However, the data from all sources points toward a reasonable degree of success in identification of StarLink™ corn, and its channeling to an approved end use (animal feed and non-human industrial products). The risk of new Cry9C corn entering the food corn supply from the 2001 and later harvests has been managed. The original estimate of EPA in the December 2000 SAP report (that U.S. corn stocks contain 0.4% uncontrolled StarLink™) now appears to be overstated, especially for corn offered for use in the food market. In fact, the Panel concludes that even Aventis' estimate of 0.125% StarLink™ corn in loads delivered to food corn processors may be too high. The Panel believes that as long as direct food corn users (dry millers, masa processors) continue to rigorously test to the lowest available

detection limits, there will be a very small and decreasing risk of producing corn based foods with detectable Cry9C protein.

(5) Corn Carry Over Patterns

Typically 13-14% of corn is carried over into the next crop year. This implies that less than 2% of corn production in a given crop year would be in the system after 2 years. This probably over-estimates actual StarLink™ carryover. While commingling within the grain system has been widely discussed, it is also true that grain handlers tend to blend-off and market old crop inventories as soon as new crop is available. Carry over inventories at the end of a marketing year likely contain less than 13-14% of the previous year's crop, because of preferential rotating done to maintain physical quality.

Carryover inventories are typically more concentrated in farm storages than in commercial storages, where turnover generates revenue. Since Aventis and USDA were more successful in recapturing corn stored on-farm relative to that commingled in commercial facilities, StarLink™ concentrations will likely decay at a faster rate than would be implied from the 13% carryover number. Producer and elevator economic incentives under the containment program will also reduce the potential StarLink™ corn carryover.

The Panel concluded that containment efforts have significantly reduced Cry9C protein concentrations in carryover stocks relative to the 1998-2000 planting frequency of StarLink™. The impact of 1998 and 1999 production on Cry9C protein levels in current U.S. grain stocks is small and rapidly diminishing. With continued testing under the GIPSA protocol, redirection of grain testing positive for Cry9C protein, producer control of volunteer corn occurrences, and removal of seed testing positive for Cry9C protein, EPA estimates that Cry9C protein will essentially be gone from corn grain in 2 to 3 years and from finished food products made from such corn in 4 to 5 years (Brassard, 2001b). The Panel concurs, although trace amounts of *cry9c* DNA may be detectable far beyond these time frames.

(6) Impact of Testing Programs by Food Processors

The potential for Cry9C protein entering the food supply depends more on the success of food processors' testing efforts than on concentration estimates of corn stocks. As long as incoming grain is consistently tested with a protocol that accurately detects Cry9C protein, corn entering the food processing system should have a maximum concentration of 20 ppb Cry9C protein. While there was considerable concern by the Panel over testing processed products for Cry9C protein, the ability to do so in raw corn has been documented by both GIPSA and the American Association of Cereal Chemists (Bridges, 2001).

It should be noted that testing by food processors is now voluntary. However, NAMA stated that once the recommendation to test was made by FDA, market forces immediately led to a high rate of testing compliance in the industry. Since the testing of

incoming grain is an important control step in preventing Cry9C protein entering the food supply, the Panel recommends that testing of incoming grain into milling operations, particularly dry milling and masa processing operations, be made mandatory at least until the 2001 corn clears the market.

The Panel would also recommend the following actions to maintain the effectiveness of exclusion of Cry9C protein from the food corn supply:

- EPA should cooperate with FDA to monitor and document positive detects.
- USDA should continue to document and audit the success of the current corn containment program.
- GIPSA/FDA should review and endorse a Quality Plan in consultation with the food corn industry.
- Success and results of the Quality Plan should be audited by an independent third party.
- All actions should be reviewed annually and updated as needed.

5. <u>Allergenic Hazard and Risk:</u>

The potential for the Cry9C protein to elicit an allergic response has been the single human health endpoint of concern for StarLink™ corn. In its December, 2000 report to the Agency, the Scientific Advisory Panel (SAP) concluded that "... there is a <u>medium</u> likelihood that the Cry9C protein is a potential allergen..." The SAP went further to recommend a number of follow- up activities that would allow for a better informed characterization of the potential allergenic risk. These activities included: (1) collection of data on the presence of specific antibodies in individuals either who claim to have experienced adverse effects after consuming food that might have contained the Cry9C protein or who have significant occupational exposure to StarLink™ corn or corn products, and (2) monitoring of reports from the medical community for individuals who claim to have experienced adverse effects either after consuming food that might have been made from StarLink™ corn or from occupational exposure to StarLink™ corn.

Question 5. FDA and CDC have been working together to investigate the adverse event reports submitted to FDA by people who claim to have had an allergic response following the ingestion of genetically modified corn products. One aspect of the investigation was to determine if these people were exposed and displayed an allergic response by the formation of serum antibodies to the foreign Cry9C protein. An FDA laboratory developed an enzyme linked immunosorbent assay (ELISA) method to detect these antibodies in the sera of the people who were potentially affected. Although there were no known Cry9C-allergic human serum samples to serve as true positive controls, the assay was able to detect reactions in sera from goats that had been purposefully sensitized against the Cry9C protein, and also to detect reactions to certain human allergens (e.g., cat, grass, peanut) in sera from humans with known allergies to these allergens.

Some of the individuals who claimed to have experienced an allergic reaction to the Cry9C protein following the ingestion of corn-based products kept samples of (or could identify) the products they ingested. FDA tested these foods for the presence of StarLink™ corn. StarLink™ corn DNA has not been detected in 10 of 11 food samples analyzed using the PCR method. The other sample of food, which tested positive using the PCR method, was not from the consumer's actual product, but from a different lot of the same product collected by FDA from a grocery store. In addition, the Cry9C protein was not detected is 9 (including the food sample that tested positive using the PCR method) of the 10 samples tested with the EnvioLogix ELISA method. One of the 10 samples tested using the EnviroLogix method was inconclusive. There was no testing of one food sample using the EnviroLogix method because there was not enough of the remaining sample to conduct the test.

a. The ability of the test to detect Cry9C-specific antibodies.

The test employed to detect Cry9C-specific antibodies is an ELISA based-antibody detection system involving the use of recombinant bacterial-derived Cry9C as a capture (coating) antigen on the ELISA plate. The test, as conducted, was done in a standard and accepted format. The laboratories have taken some effort to increase the chance of signal detection by coating the wells with a maximum amount of antigen and using a low dilution of the serum samples being tested. However, there are a number of limitations and/or problems associated with the assay.

Importantly, the quality of the test cannot be fully determined given the lack of positive control sera (serum from patient[s] with known Cry9C allergy possessing anti-Cry9C IgE antibodies). Utilizing sera from populations with high exposure to StarLink™ corn (e.g. occupationally exposed employees such as feed mill workers) might be helpful in locating positive control sera.

Additional secondary detection systems (e.g. chemiluminescence, fluorescence, immunoblotting, and avidin/biotin-enhanced detection systems) with greater sensitivity should be considered. The validation of the current method to detect antigen-specific IgE is based upon the ability of the assay to detect IgE antibodies to whole peanut, cat and grass pollen [possessing multiple proteins] as opposed to antibodies to a single protein. The employment of whole allergen extracts for capturing IgE is expected to produce a higher sensitivity (due to increased number of epitopes) than using a single protein (as has been done for the Cry9C assay). Consequently, the Panel is concerned that the current assay lacks sufficient sensitivity to conclude that the sera tested contained no Cry9C-specific IgE antibodies.

The test, as conducted, is limited by the use of *E. coli*-generated Cry9C, which may not possess the same epitopes as the Cry9C protein expressed in StarLink™ corn. There is no assurance that bacteria-derived Cry9C is properly folded; in other words, the protein may not be in the right conformation for antigen detection. Although the bacteria-derived Cry9C is reportedly biologically active (e.g., with regard to pesticidal

activity), it is not clear that the appropriate conformational epitopes are present with this protein.

Since IgG anti-Cry9C antibodies are likely to be produced at higher concentrations than IgE antibodies, the IgE response may be masked by antigen-specific IgG. Quantification of Cry9C specific IgG, or depleting the serum samples of IgG by appropriate techniques, may enhance the reliability of specific IgE detection.

The use of only a single dilution of serum (1:2) for detection of Cry9C-specific IgE raises concern about the potential effect of serum inhibitor(s), reinforcing the need for testing lower serum dilutions.

It would be of value to establish that human IgG is readily detectable in the current assay. Since many antigenic and allergenic epitopes are shared, it would confirm that the appropriate epitopes are displayed.

b. The criteria used to designate test results as positive or negative, and the significance of positive and negative results obtained using this test.

The criteria employed to determine a positive result is a signal that is greater than 2.5-fold compared with the background signal. This is a relatively sensitive criterion for determining a positive result, and is appropriate when attempting to design an assay of maximum sensitivity. The criteria for negative results are based on no significant increase in the optical density [O.D.] compared to negative control wells (containing buffer and no sera), sera from atopic individuals (with elevated IgE antibody levels) and banked sera that pre-dated introduction of StarLink™ corn. The negative result is weighted against positive results with goat anti-Cry9C IgG, generated by immunization with bacteria-derived Cry9C in complete Freund's adjuvant, and positive results with ELISA assays for IgE antibodies to whole grass pollen, cat, and peanut extracts. While these positive controls are helpful, the goat-derived IgG antibodies are generated by methods that are unlikely to mimic immune sensitization to natural Cry9C in humans, and the aero- and food-allergen-specific assays detect IgE to multiple proteins comprising the allergen. Taken together, the negative results are significant in that they reduce somewhat the likelihood that IgE-mediated allergic reactions are responsible for the symptoms observed, but the assay lacks sensitivity and specificity (to other delta endotoxins) to exclude the presence of Cry9C-specific IgE and the possibility of allergic reactions to the protein.

c. The ability of the test to either identify or eliminate Cry9C as a potential cause of the allergic symptoms reported

The test, as conducted, does not eliminate StarLink™ Cry9C protein as a potential cause of allergic symptoms. The negative results decrease the probability that the Cry9C protein is the cause of allergic symptoms in the individuals examined. However, in the absence of a positive control and questions regarding the sensitivity and specificity of the assay, it is not possible to assign a negative predictive value to this

finding. The use of non-equivalent, bacteria-derived coating antigen raises the possibility that IgE directed against plant derived Cry9C may not be detected. Given the lack of information on the specificity of the assay to detect StarLink™ corn-derived Cry9C, the assay does not eliminate the possibility that the individuals possess IgE antibodies to Cry9C and reacted to the StarLink™ corn.

Additional studies are necessary to eliminate Cry9C proteins as a potential cause for the allergic symptoms reported. These studies should include a sensitive and validated assay to determine serum levels of IgE-specific to corn Cry9C protein, skin testing with water soluble extracts of StarLink™ corn and possibility double blind placebo control food challenge (DBPCFC).

d. The usefulness of the test, along with other information gathered in the FDA and CDC investigation, in evaluating whether an individual has experienced an allergic reaction to the Cry9C protein

The ELISA test (developed by FDA) provides a useful first level approach to examining allergic responses to bacterial Cry9C. The negative results must be viewed in light of the potential problems associated with the assay, as outlined above. It therefore remains critical to perform a second-level, more sensitive and specific analysis for IgE anti-Cry9C. Immunoblotting with StarLink™ and non-StarLink corn with sera from individuals suspected of reacting to StarLink™ corn would provide the next level of screening for Cry9C allergic responses. The immunoblotting should be more sensitive and would also identify specific protein bands recognized by the sera.

The PCR test, designed to screen food for the presence of the cry9c gene, is a useful screen for the possible presence of StarLink™ corn, but without standardization (e.g., quantification with regard to a standard), it is difficult to assign a negative predictive value to this result. A validated quantitative PCR test should be used in a head-to-head comparison with the ELISA so that it can be established whether a negative PCR result is more sensitive than a negative ELISA result.

The Panel discussed the need for appropriate sample size to adequately assess whether allergic reactions have occurred to the Cry9C protein. This is presented in response to question 9. In conclusion, the Panel believed that the results presented somewhat lessen the likelihood that the individuals examined experienced an allergic reaction to StarLink™ corn Cry9C protein, but these results do not eliminate the possibility of such a reaction.

6. In the December, 2000 SAP report, after reviewing the information then available concerning the Cry9C protein, the Panel concluded that "... there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of the Cry9C protein itself..." The same report went on to state that "Given the current state of knowledge regarding allergens and the uncertainties of ascertaining the exact amounts of Cry9C in the food chain, this approach [collecting data on the presence of specific antibodies in individuals claiming exposure to

Cry9C in food products] could provide "hard evidence" as opposed to speculation on the question at hand." Since then, additional information concerning the potential allergenicity of the Cry9C protein has become available, including the FDA/CDC report issued on June 11, 2001, which provides information on the presence of Cry9C-specific antibodies in individuals claiming to have experienced an allergic reaction after eating corn-based foods. In light of the available information, what is the current Panel's view on the previous finding of that there is a "medium likelihood" that Cry9C protein is a human allergen? Please comment specifically on whether and how that view is significantly affected by your consideration of the June 11, 2001 reports from FDA and CDC.

The Panel agreed that new data concerning the StarLink™ corn protein, including the FDA/CDC report of June 11, 2001, which provided information on the detection/non-detection of Cry9C-specific antibodies in individuals reporting an allergic reaction after eating corn-based foods, have not substantially increased the understanding of the allergenic potential of the Cry9C protein. The Panel had no reason to adjust the SAP's previous conclusion as cited: "The Panel agreed that there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of Cry9C protein itself - not its levels in the food" (SAP Report No. 2000-06, page 10).

The following briefly summarizes the weight-of-the-evidence and the basis for the Panel's decision. Based on the data submitted since the last SAP meeting, with respect to the allergenic criteria listed as items #1 through #6 as presented in SAP Report No. 2000-06, page 10, no new evidence has been presented that demonstrates StarLink™ Cry9C's protein allergenic potential is *diminished*. Further data have not yet been presented that resolve the question of *substantial equivalence* between bacterial derived Cry9C, whether isolated from recombinant DNA produced in *Escherichia coli* or *Bacillus thuringiensis* strains, and Cry9C protein in StarLink™ corn. As reported by previous SAPs on this subject, (SAP Report No. 2000-01A, page 12): "it is difficult to accurately evaluate the intrinsic potential of a protein to provoke an IgE antibody response to cause allergic sensitization. There is no known amino acid sequence or motif that contributes to a protein being identified as a potential allergen". Despite these limitations, data should have included the full linear amino acid sequences of proteins under consideration. Information in the Aventis amino acid sequence homology study MRID #442581-09 and in data presented in the recent Aventis study (96QZM007) are inadequate to deduce the full amino acid sequence homology as previously recommended by the SAP Report No. 2000-07, page 75. From the latter study, the Panel can only conclude that the N-terminal amino acid sequence of plant-derived Cry9C is slightly different from the bacterial produced Cry9C. Specifically, the plant-derived Cry9C includes an added methionine and alanine at the N-terminus.

The Panel is concerned about the appearance of the 55kDa fragment. It is not clear whether this fragment is derived from a partial degradation in bacteria or corn plant, is generated during purification, or is caused by degradation by the resident intestinal flora. This was previously raised by the Panel (SAP Report No. 2000-01A, page 8). There is a lack of definitive data to demonstrate that there are identical fragments

generated upon ingestion of StarLink™ corn compared to the "natural degradation" observed in the various bacterial preparations that have been used to immunize animals for sources of anti-Cry9C specific IgG antibody production.

Aventis has reported that the Cry9C protein appears not to be glycosylated in corn (*MRID # 443844-01*). The last SAP (SAP Report No. 2000-06, page 11), questioned this statement. The latest data of Aventis study 96QZM007 also states: "It is concluded that applying the DIG-periodic acid staining method, no extensive glycosylation of the Cry9C protein is detectable". The Panel found that the experimental approach may not have been adequate to detect low levels of glycosylation of the protein. To resolve this issue, the Panel recommends that a more sensitive method for the detection of glycosylation should be used or alternative methods be used to verify carbohydrate content.

Thus, there are still questions about the scientific validity of using the bacterial derived Cry9C protein in the hazard assessment of allergic potency. This includes its use as an immunogen to stimulate an immune response, e.g., to immunize animals in order to produce polyclonal IgG antibodies; and secondly, as the antigen to capture antibodies via structure complementary to its receptor site, e.g., solid-phase immunoassays.

There is no reason to withdraw or alter the conclusion of the last SAP of November 28, 2000, "Given the current state of knowledge regarding allergens and the uncertainties of ascertaining the exact amounts of Cry9C in the food chain, this approach (detection of specific IgEs in sera of food-diseased people) could provide 'hard evidence' as opposed to speculation on the question at hand." IgE is the single antibody class that serves as a reliable marker for the induction of an immediate hypersensitivity (allergic reaction) in humans. The Panel remains convinced that any other antibody reactivity (IgA , IgG1 or IgG2) would be of relatively little use in the verification of immediate-type I allergic reactions (SAP Report No. 2000-06, page 17/18). FDA and CDC have therefore taken a logical step in assessing the presence of possible Cry9C-specific IgE antibodies in those individuals who reported an adverse health effect upon eating corn derived foods potentially containing StarLink™ Cry9C protein. Evaluations of banked serum samples submitted to CDC and analyzed by FDA's method for detection of IgE antibodies to Cry9C, did not detect anti-Cry9C IgE in the serum of individuals reporting adverse reactions. The Panel concluded it was less likely that individuals submitting their sera reacted to Cry9C protein. However, given the concerns over the validation of the ELISA assay (lack of a positive control; use of *E. coli*-derived Cry9C; lack of sensitivity) the Panel cannot eliminate the possibility that the reactions were compatible with a reaction to StarLink™ Cry9C. Such uncertainty should be addressed by employing Western immunoblots using serum from subjects reporting adverse reaction comparing the IgE binding of both non-StarLink™ corn and StarLink™ corn kernel extracts.

Investigating serum from exposed populations, such as agricultural workers, grain and feed mill workers, and seed company employees as potential sources for serum IgE reactive to StarLink™ Cry9C remains a priority. In addition, the number of individuals reporting adverse reactions to FDA/CDC remains small, and continued examination of new reports of adverse reactions to corn should continue. The Panel heard from two

private citizens who reported adverse reactions to eating corn products that potentially contain StarLink™ Cry9C. The Panel recommends that every attempt should be made to follow up these two individuals with skin testing, serum IgE analysis, and if agreeable a double blind placebo control food challenge to ascertain that the adverse reaction was indeed the result of eating StarLink™ corn products.

To date, additional questions submitted by previous SAP reports remain unanswered. These include: Do the IgG antibodies produced in mouse, rabbit, and goat against bacterial expressed Cry9C detect StarLink™ expressed Cry9C? It is thus further recommended that additional denaturation/degradation experiments such as those provided in the immunoblot analysis of rabbit anti-Cry9C binding to enzymatically digested Cry9C proteins be performed.

To support the collection of hard evidence on antigen-specific IgE-levels, it is important to note the need for validated methods for analyzing StarLink™ Cry9C-derived protein levels in processed foods and intermediates as distinct from the PCR methods used to detect DNA in foods (SAP Report No. 2000-06; EPA, 2001; CDC, 2001a/b).

The Panel recognized the combined work and efforts of members of the EPA, FDA, and CDC in their data collection regarding: 1) confirmation of the adverse event effects reported by individuals related to the ingestion of corn products; 2) collection of incriminating food samples where possible for Cry9C detection; and 3) collection of serum samples and testing for Cry9C-specific IgE. However again, the technical approach for the detection of Cry9C protein and antigen-specific IgEs is limited and cannot resolve the issue of the presence or absence of Cry9C-specific IgE in the serum of individuals reporting adverse reactions after eating corn.

7. In its December 1, 2000 report, the Panel concluded that "…the likely levels of Cry9C protein in the U.S. diet provide sufficient evidence of a low probability of allergenicity in the exposed population."

a) In light of the new information on the levels of Cry9C protein in the diet and the other available information concerning potential allergenicity, please comment on the overall probability that the likely levels in the US diet of Cry9C protein are sufficient to cause significant allergic reactions in a major identifiable subgroup of the exposed population. To the extent permitted by available information, please characterize the current level of potential risk in terms of the proportion of the population likely to be affected and the nature and severity of potential effects.

During the SAP meeting, new information [some provided prior to the meeting] was presented on the general population's potential level of exposure to Cry9C. However, the Panel raised a number of questions on the reliability of these calculations, which were based on an ELISA to detect Cry9C protein in various finished food products. While the ELISA used to quantitate Cry9C in food proteins suggested very

limited exposure to Cry9C (<20 ppb), there are significant questions about the ELISA test for finished foods.

The ELISA, as presented, is confounded by two factors. First, bacterial-derived recombinant protein rather than the authentic natural plant-derived StarLink™ protein was used as the capture antigen. It remains possible that the bacterial-derived protein is not identical to the plant-derived protein (as shown previously by the differences in molecular weights and by differences in glycosylation of the two proteins). Thus, the antibodies generated against the recombinant bacterial protein may not recognize all epitopes present in the plant-derived protein. In addition, this deficiency may be magnified by the fact that the sandwich ELISA utilized uses the same antibody for both capture and detection antibody-conjugate. Second, the antisera employed has not been appropriately characterized with respect to its recognition of denatured or degraded Cry9C protein, both of which are likely to be present and potentially allergenic in processed food. The new data presented concerning the ability of the antisera used in various ELISAs to detect Cry9C peptides were very limited. One immunoblot, shown by Aventis, was limited to the analysis of Cry9C protease fragments, not degraded and/or denatured products generated during food processing. The statement that the antisera recognizes all Cry9C degradation products is not justified by the data shown and seems implausible based on immunogenicity studies with other proteins. Additionally, an appropriate analysis of recognition of denatured Cry9C by the antisera has not been presented. Given these concerns, the new data on Cry9C protein levels in the diet remains problematic, and thus no convincing evidence was presented to change the current view on allergenicity. Given the questions raised about this assay and the data generated from it, the Panel would again conclude that "*the likely levels of Cry9C protein in the U.S. diet provide sufficient evidence of a low probability of allergenicity in the exposed population*." The importance of informing the medical communities for surveillance is covered under Question #8.

In addition, the subpopulations most likely to be affected, including young infants placed on hypoallergenic formulas and children with multiple food allergies who are most susceptible to developing an allergic response to the Cry9C protein because of their high corn consumption, have not been addressed. The recommendation to involve the allergy community for surveillance of this problem was not implemented.

b) If you conclude that it is probable that the expected levels of Cry9C protein are sufficient to cause significant allergic reactions in a major identifiable subgroup of the exposed population, please identify a level of Cry9C protein below which you would not expect significant reactions to occur in a major identifiable subgroup of the exposed population.

There are no reliable data on threshold levels of isolated food proteins for inducing allergic responses in highly sensitive patients. Some data have been generated on whole foods, i.e., a composite of proteins, such as milk or peanut. In addition, much of the data on reactivity to whole foods, e.g., peanuts, have not been derived from the most highly sensitized patients. Yet another problem in defining a threshold level for

Cry9C protein is the lack of confidence in the ELISAs to detect Cry9C protein in finished food products. Although the Panel discussed the potential threshold Cry9C levels in raw corn and finished food products, it concluded that there was insufficient scientific data to support recommending any threshold values.

c) Based on your response to questions 7a) and 7b), do you conclude that there appears to be a maximum level of Cry9C protein for which, if that level were found in corn grain and foods made from such grain, there would be a reasonable scientific certainty that exposure would not be harmful to public health? Please explain your answer.

Based on the Panel's response to question 7b, the Panel could not determine a threshold level of Cry9C protein where there would be a reasonable scientific certainty that exposure would not be harmful to public health. No reliable data are available on threshold levels of isolated food proteins for inducing allergic response in highly sensitive individuals. Thus, the Panel concluded that based on *reasonable scientific certainty,* there is no identifiable maximum level of Cry9C protein that can be suggested that would not provoke an allergic response and thus would not be harmful to the public.

Possible Need for Additional Data and Additional Public Health Measures:

Question 8. In its December 2000 report, the SAP concluded "...the Agency should place ...priority on monitoring of reports from the medical community. The Panel felt that the medical community should be informed of the investigation into the allergenicity of Cry9C in corn products". Approximately 8 months have passed since that original recommendation and, given the materials that have been discussed at today's meeting, we ask the Panel to please comment on the value of implementing a program involving the medical community intended to detect instances in which individuals experienced allergic reactions to the ingestion of Cry9C protein in food. If the Panel still regards such a program as potentially valuable, then please comment on the scope and design of such a program.

The Panel agreed that the passive surveillance program now in place should be continued. A program involving the medical community should be initiated as a part of this surveillance effort to further define instances/cases in which individuals experienced an adverse reaction in association with the ingestion of StarLink™ corn containing food. The value of this program would benefit two essential groups: (1) individuals with possible adverse events and (2) the public at large. The individual would benefit by identification or dismissal of a specific food allergy to StarLink™ Cry9C protein; thus allowing the appropriate cause of the adverse event to be identified. The public would benefit from assurance of the safety of the food supply. The Panel felt the surveillance effort should be continued for two years. After 2 years, the program should be re-evaluated to determine if it should be continued.

The scope of the program would include the following:

- The inclusion of government entities with relevant expertise.
- The dissemination of information to allergists and primary care physicians, including members of the American Academy of Allergy, Asthma and Immunology and the American College of Allergy, Asthma, and Immunology. Physicians should be encouraged to report adverse food reactions to the FDA. The EPA should continue to work with the FDA to identify individuals with reported allergic reactions to StarLink™ Cry9C. The program should be established to determine whether these reactions are in fact due to Cry9C using the criteria outlined in this report.
- Consideration should be given to initiating a clinical study of StarLink™ corn exposed populations (e.g., children, farmers and their families, grain and mill workers, and other occupationally exposed groups) to identify individuals (if any) experiencing allergic reactions to StarLink™ corn. In this study, the following approaches should be employed:

(1) Study participants should give informed consent.
(2) A detailed history and physical examination should be obtained on those individuals reporting a possible allergic to StarLink™ corn.
(3) Additional tests for food allergy in those with a history compatible with an allergic reaction to StarLink™ corn should include skin testing with water soluble extract of StarLink™ corn, determinations of the presence of anti-Cry9C specific IgE, and a DBPCFC using validated reagents/protocols.
(4) If individuals are identified who are sensitive to StarLink™ corn, they must be instructed on avoidance and instruction on how to treat inadvertent exposure.

Beyond the response to question #8, the Panel recommends that the CDC establish a monitoring system for suspected severe allergic reactions to food.

One member of the Panel recommended a random sampling of serum from a large cohort of normal exposed individuals for determinations of anti-Cry9C IgE. Others in the Panel questioned whether this approach would yield useful information.

9. In its December 2000 report, the SAP identified additional types of information that could improve EPA's ability to assess the potential allergenic risk to humans from Cry9C protein in the food supply. In response to the Panel recommendations, Aventis Crop Science and the Federal government have developed new information on the Cry9C protein, which has been presented to the Panel today. Given all the information that we presently have, please characterize generally the adequacy of the existing scientific database to evaluate the allergenic risk of Cry9C and identify any additional information that would be feasible to generate and would be likely to change significantly the current assessment of the allergenic risk to humans from the Cry9C protein in the food supply.

The available database has given the Panel no reasons to adjust the SAP's previous hazard assessment "...that there is a medium likelihood that the Cry9C protein is a potential allergen based on the biochemical properties of Cry9C protein itself - not its

levels in the food" (SAP Report No. 2000-06, page 10). Anticipated risk of Cry9C protein being an allergen remains largely unchanged based upon the data presented, and the Panel continues to conclude that the risk of exposure adequate to induce sensitization is low. The Panel provided a more detailed explanation in its response to question #6.

The Panel agreed that it is not possible to assign an absolute threshold level for preventing a food or food-protein-induced allergic reaction, since the existing database does not contain sufficient scientifically sound information to establish a threshold level for the Cry9C protein, as addressed in Question #7. The new data made clear that for a certain time period, trace levels of Cry9C protein are likely to continue to be unavoidably present in food products prepared from domestic yellow corn, although at diminishing levels.

The Panel restated that the risk of allergic reaction to the Cry9C protein can only be ascertained through thorough evaluation of confirmed case reports of adverse reactions to ingestion of foods, which should include complete history, validated laboratory examination and in most cases, DBPCFC performed under appropriate medical supervision.

As stated by the November 28th SAP, human allergenic risk is linked to exposure. The Panel thus reevaluated the exposure assessments relating to human food produced from yellow 'dent' corn and whether it contributed significantly, if at all, to potential human exposure to Cry9C protein. The Panel concluded that the exposure risk to StarLink™ corn is decreasing and thus supports the containment efforts by Aventis, USDA/GIPSA and others that began in October of last year. Indeed, the Panel believed that if procedures in place continue, future human exposure to the Cry9C protein in yellow corn-derived food products would be significantly less than the EPA's November, 2000 calculations. Data presented from sera obtained from individuals reporting reactions to corn products provided no evidence of detectable Cry9C-specific IgE, which would suggest no sensitization to Cry9C from StarLink™ corn. However, the Panel questions the sensitivity and specificity of the current ELISA utilized to evaluate sera from these subjects. Again, it should be noted that the DBPCFC is the only way to confirm the diagnosis by establishing a cause-and-effect relationship.

The following briefly summarizes the Panel's recommendations for additional information (the recommendations are not presented in an order of priority):

- More biochemical data are needed such as full-length amino acid sequence analysis, a more sensitive methodology to analyze post-translational modifications, standard inhibition curves in order to differentiate the analyte Cry9C from analogues of the compound [e.g. native, denatured, and fragments generated under realistic processing conditions]. Data should be provided that would support the use of the bacterial-derived Cry9C protein in the hazard assessment of the allergenic potential. Evidence is needed to verify that epitopes of unprocessed and processed corn-derived Cry9C are comparable to those present on the bacterial-derived protein.

- The EPA should work with other Federal agencies, including FDA, in validating the ability of current immunoassays to detect StarLink-produced Cry9C in processed foods. This is necessary to determine the extent to which we can interpret the data, suggesting a reduction in Cry9C protein levels resulting from food processing.
- Similar to the preceding statement, EPA should work with other Federal agencies, including FDA, to validate the diagnostic sensitivity and specificity of the existing ELISA used to identify anti-Cry9C antibodies. The Panel concluded that there is a risk of false negative results because of the design chosen. The CDC should obtain more serum samples from individuals suspected of reacting to corn products, and these samples should be examined for the presence of IgE and IgG antibodies to StarLink-produced Cry9C [use of the immunoblot technique may facilitate this process]. The presence of Cry9C-specific IgG antibodies, which often bind to the same epitopes as IgE antibodies, would demonstrate that the capture antigen utilized, i.e., the *E. coli* -generated Cry9C, displays epitopes representative of the corn-derived Cry9C.
- The Panel believed that it would be useful to develop an appropriate Cry9C extract for use in skin prick testing of patients reporting suspected reactions to StarLink™ corn.
- In order to come to the correct diagnosis of a Cry9C-allergic reaction in individuals claiming sensitivity to StarLink™ corn, the investigative process of spontaneously reported cases should be continued. DBPCFCs will be necessary to answer the question: Is the reaction reproducible? (SAP Report No. 2000-06, page 17).
- In order to have sufficient statistical power to state [with reasonable certainty] that no reactions have occurred to the StarLink™ corn, the Panel felt that at least 30 patients with suspected reactions to “suspect” corn products should be investigated using validated ELISAs, immunoblots, and possibly DBPCFCs.
- EPA should notify the allergy community of the possible allergenicity of the Cry9C protein in corn products.
- Studies to establish the presence [or absence] of Cry9C-specific IgE and IgG antibodies in individuals who have significant occupational exposure to StarLink™ corn or derived products would be extremely useful in establishing the antigenicity/allergenicity of the Cry9C protein. The best course would be to collect sera, for example, from exposed workers, as has been recommended by one of the previous SAPs or, from workers of the feed milling industry.

Question 10. From a public health perspective, please identify other measures, if any, beyond those currently being implemented that you consider feasible and necessary to reduce the likelihood that people would experience allergic reactions from ingestion of food containing Cry9C protein.

- Establish the appropriate protein equivalence standards for all studies on StarLink™ corn.
- Require the use of the commercial "Lateral Strip Test" in dry milling facilities.

- Increase the capture of cases of possible allergy to StarLink™ corn. This would include conducting outreach through educational programs for the public and the medical community to increase awareness of adverse reactions to StarLink™ corn.
- Continue programs designed to identify and remove StarLink™ corn from the food supply.
- Verify corn and seed not involved in voluntary recalls are free of StarLink™ corn; if not, establish an approach to address the removal of StarLink™ corn from these sources.

One Panel member considered labeling products as "may contain" StarLink™ corn since consumers would then be alerted to the possible presence of Cry9C. Without labeling, there would be no basis for consumers to recognize that a given corn product is different from that produced from non-Cry9C containing corn.

Question 11. Are there any other comments on the science of this issue that EPA should consider or that the SAP panel would like to address?

The EnviroLogix ELISA analytical testing procedure must be validated for further use by resolving the apparent protein solubility and processing issues in food products prepared from dry-milled and masa-processed corn. This involves the apparent large, unaccounted loss of protein during the preparation of food items such as hush puppies, corn muffins, polenta, corn puffs, "ringed" cereal, soft tortillas, fried tortilla chips, and baked taco shells (Figure 5, page 43, Aventis Report B003244, Volume 5 of 7). Protein is definitely not destroyed under such conditions but can be converted to an insoluble or denatured form that could interfere with its extraction and detection by this analytical procedure.

A critical retrospective review needs to be undertaken by an independent, balanced ad hoc committee concerning the scientific issues involved with StarLink™ corn. The events surrounding the approval and subsequent health issues relative to StarLink™ corn offer an experience from which much can be learned. What are the scientific issues undergirding the origin and continuation of the issue? What went right? What went wrong? What have we learned? For example, a split registration for feed use only did not work. How did Cry9C penetrate the human food supply? Why was the adulteration detected by a public interest group rather than through a more formal surveillance program (e.g., Federal agencies or regulated industry)?

Aventis appears to have furnished virtually all samples for the current evaluation. Is this appropriate? The Panel favors establishment of a procedure to independently validate reagents and materials.

More resources and attention need to be focused on the fields of food allergy and allergenicity. It is amazing how little we know about many aspects and facets of allergen issues. What makes a protein allergenic? Tests are needed for identification of such agents. Programs are needed to train new investigators for this area. Governmental and

private sector programs are limited by funding and should be better supported. Sufficient and adequate information is needed to establish thresholds or tolerances.

There is a need for the issues of allergenicity to be more fully developed in the context of genetically improved crops and foods. This should include review of other guidelines, such as the WHO decision tree for allergenicity of GMO foods, for consideration of these issues and corresponding decision making. Risk:benefit ratios should be investigated and established for genetically improved crops and foods with accompanying recommendations. The issues and evolving importance of genetic modification of agricultural commodities and foods warrant continued review.

When FDA requested industry to provide information concerning reports of potential allergenic responses, much of the data was aggregated. This was of little use to the FDA/CDC in initiating follow-up contacts and interviews. Procedures should be developed and supported for follow-up investigation of such reports, taking into account regulatory/legal constraints.

The grain industry has reacted to effectively deal with the issues of StarLink™ corn, its containment, and redirection to feed use so that it does not enter the human food chain. It has been sufficiently effective in this regard, that the Panel recommends that some StarLink™ corn be secured and stored for future studies.

During discussions of exposure analysis, it became apparent that current food intake surveys are not particularly useful in establishing food intake over a several consecutive day period. There is a need for longitudinal data to allow this to be done.

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